THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 63: 476–478, 1974

Vol. 63, No. 6
Printed in U.S.A.

CHROMATOGRAPHIC FRACTIONATION AND PARTIAL CHARACTERIZATION OF ACID PHOSPHATASE IN GUINEA-PIG EPIDERMIS*

TERUO MIYAGAWA, M.D., HIROMU KODA, M.D., AND HARUKUNI URABE, M.D.

ABSTRACT

Two chromatographically and enzymatically distinct forms of acid phosphatase have been separated by gel filtration from an extract of guinea-pig epidermis. These enzymes, termed $APase_1$ and $APase_2$, had pH optima for *p*-nitrophenyl phosphate hydrolysis around 5.0. $APase_1$ was inhibited by fluoride, L(+) tartrate, and lead nitrate, but was insensitive to formaldehyde and glutaraldehyde. In contrast, $APase_2$ was insensitive to fluoride, L(+) tartrate, and lead nitrate, but was sensitive to formaldehyde and glutaraldehyde. K_m values for $APase_1$ and $APase_2$ with *p*-nitrophenyl phosphate as substrate were 0.09 mM and 0.125 mM, respectively.

Although many biochemical, histochemical, and electron microscopical studies of epidermal acid phosphatase [1–12] have been done, it has not been clarified whether or not epidermis has more than one such enzyme. Acid phosphatase activity is observed principally in the transition zone of the epidermis (stratum granulosum and stratum corneum) [4,5]. Braun-Falco and Rupec [11] found that acid phosphatase is localized to several sites in human epidermis, but little is known about the biologic function of the enzyme. As the first step in studies of the biologic function of the enzyme, we have examined the epidermal enzyme for heterogeneity.

This paper demonstrates the presence of two forms of acid phosphatase in guinea-pig epidermis and documents the differences in their properties.

MATERIALS AND METHODS

Extraction of enzymes from guinea-pig epidermis. Male adult guinea pigs (300–400 gm) were killed by stunning and exsanguination. Hair was plucked from the back and epidermis was obtained by the stretch method. An homogenate (2 min at 1000 rpm) was prepared in an all-glass homogenizer with with 0.1 M sodium acetate buffer, pH 5.0, containing 0.2% Triton X-100 (100 mg moist weight per ml). The homogenate was centrifuged for 15 min at 10,000 × *g*, and the resultant supernatant fraction was again subjected to centrifugation at 10,000 × *g* for 15 min. These and all subsequent procedures were carried out at 4°C if not indicated otherwise.

Chromatography on Sephadex G-100. Sephadex G-100 columns (48 x 2.8 cm) were prepared and equilibrated with 0.1 M sodium acetate buffer, pH 5.0. Two milliliters of the supernatant solution were applied to the column and eluted with 0.1 M sodium acetate buffer, pH 5.0, at a flow rate of 0.5 ml per min. Fractions of 10 ml were collected, and the protein content was estimated by absorption at 280 nm.

Measurement of enzymic activity. Acid phosphatase activity was measured at 25°C at pH 5.0 by the rate of liberation of *p*-nitrophenol from *p*-nitrophenyl phosphate. The routine assay mixture (1.0 ml) contained 0.7 ml of 0.1 M sodium acetate buffer, pH 5.0, 0.2 ml of eluate ($APase_1$, fraction no. 11; $APase_2$, fraction no. 18; see Fig. 1), and 0.1 ml of 13.5 mM *p*-nitrophenyl phosphate. After incubation for 20 min at 25°C, the reaction was stopped by the addition of 2 ml of 0.2 N NaOH. The extinction of the liberated *p*-nitrophenol was read at 410 nm against a zero time control. No measurable breakdown of the substrate occurred in the absence of the enzyme at any pH used. An enzyme unit was defined as a 0.1 optical density increase at 410 nm for 20 min under the above assay condition.

Sephadex G-100 was purchased from Pharmacia (Sweden), and all other reagents were of the purest grade commercially available. Substrate and inhibitor solutions were prepared in 0.1 M sodium acetate buffer, pH 5.0, and the pH was readjusted to 5.0, if necessary, with small volumes of acid or base.

RESULTS

The present study showed differences in the physical and enzymatic properties of the two acid phosphatases of guinea-pig epidermis separated by gel filtration. These included differences in pH optima, inhibitor, and thermal inactivation as well as K_m values.

Distribution of acid phosphatase during chromatography. Figure 1 represents a typical elution pattern obtained when the 10,000 × *g* supernatant fraction of an epidermal homogenate was chromatographed on Sephadex G-100. Two peaks of acid phosphatase activity were separated by this procedure, and these will be referred to as $APase_1$ and $APase_2$.

Optimal pH. The optimal pH for $APase_1$ and $APase_2$ were determined in 0.1 M sodium acetate buffer (pH 4.0–6.0), 0.1 M potassium phosphate buffer (pH 6.0–7.0), and Tris–HCl buffer (pH 7.0–10.0). Optimal pH for $APase_1$ and $APase_2$ were 5.0 and 5.25, respectively. A difference in the shape of the pH activity curves was observed. A sharp peak of maximal activity was observed for $APase_1$, and a broad region of maximal activity, pH 4.75 to 6.0, was observed for $APase_2$. The presence of

Manuscript received April 8, 1974; in revised form June 24, 1974; accepted for publication June 26, 1974.
* From the Department of Dermatology, Kyushu University School of Medicine, Fukuoka 812, Japan.

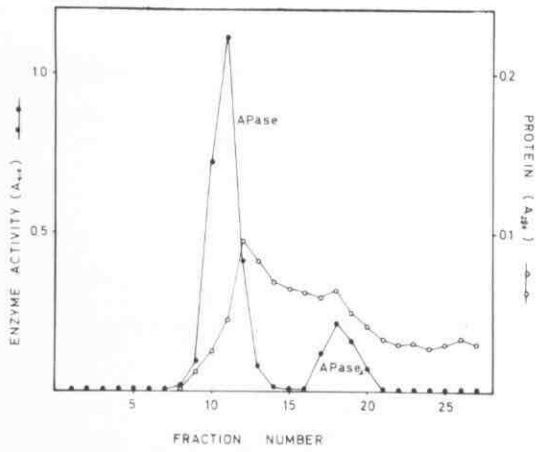

FIG. 1. Elution pattern of epidermal acid phosphatases from Sephadex G-100 column.

Mg^{++} did not affect either $APase_1$ or $APase_2$ activity. There was no APase activity between 7.5 and 10.0.

Thermal inactivation. The two preparations were incubated for 10 min at temperatures ranging from 25° to 60°C, and subsequently the hydrolysis of *p*-nitrophenyl phosphate was determined. Figure 2 shows the percentage of activity remaining after heating as compared with the untreated control. It is clear that there are two thermolabile acid phosphatases in guinea-pig epidermis. The mean temperature at which 50% inactivation of each enzyme occurred under these conditions was approximately 41°C for $APase_1$ and 53°C for $APase_2$.

Inhibition studies. Various concentrations of fluoride, L(+) tartrate, lead nitrate, formaldehyde, and glutaraldehyde (Tab.) were tested for their effects on the hydrolysis of *p*-nitrophenyl phosphate by $APase_1$ and $APase_2$. These studies showed that $APase_1$ was sensitive to fluoride, L(+) tartrate, and lead nitrate, whereas $APase_2$ appeared resistant. In contrast, $APase_2$ was inhibited by formaldehyde and glutaraldehyde.

K_m values. The K_m of each enzyme for the substrate (*p*-nitrophenyl phosphate) was determined at pH 5.0. The K_m values for $APase_1$ and $APase_2$ were 0.09 mM and 0.125 mM, respectively.

DISCUSSION

Although multiple forms of acid phosphatase differing in their properties and intracellular distribution are known to exist, there is no report demonstrating the heterogeneity of epidermal acid phosphatase. Braun-Falco and Rupec [11] used electron microscopy to document that acid phosphatases existed in both free and bound forms. The latter was bound to structural elements; such as keratohyaline granules and lysosomes in human epidermis. Biochemical studies of this enzyme in the epidermis have been performed, but these data

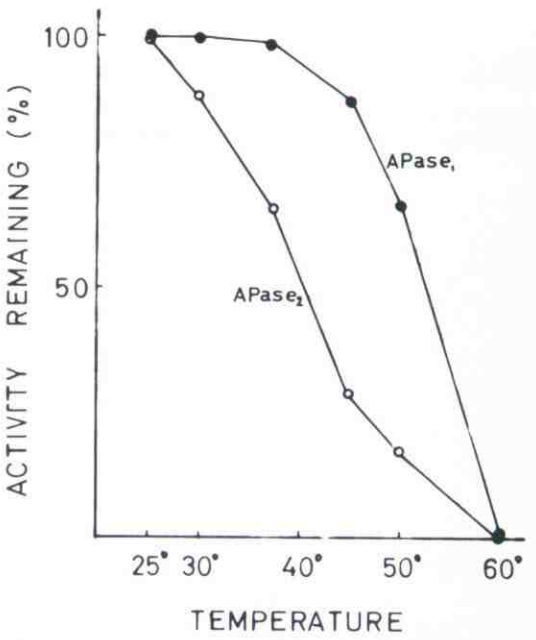

FIG. 2. Thermal inactivation of the two epidermal acid phosphatases. The enzy nes were incubated in 0.1 M sodium acetate buffer, pH 5.0, for 10 min at the indicated temperature. After the reaction mixtures had been cooled in an ice bath, *p*-nitrophenyl phosphate was added, and the remaining enzyme activity was determined. The results are given as precentages of the unincubated control value for each enzyme.

TABLE

Effect of various concentrations of fluoride, tartrate, lead nitrate, formaldehyde, and glutaraldehyde on p-nitrophenyl phosphate hydrolysis by the two enzyme preparations

Compounds	Conc.	Inhibition (%)	
		$APase_1$	$APase_2$
Control		0	0
Fluorine	0.1 mM	60	1
	0.5 mM	81	2
	1.0 mM	83	9
L(+) Tartrate	0.1 mM	33	1
	0.5 mM	57	2
	5.0 mM	91	4
Lead nitrate	0.05 mM	10	0
	0.5 mM	28	1
	1.0 mM	54	2
Formaldehyde	0.05%	2	58
	0.1%	3	72
	0.25%	10	90
Glutaraldehyde	0.5%	8	24
	2.5%	5	48
	5.0%	5	68

did not demonstrate heterogeneity of epidermal acid phosphatase.

DiPietro and Zengerle [13] showed that three distinct forms of acid phosphatase from human placental tissue (Sephadex G-200) differed in size and were also enzymatically distinct. The placental acid phosphatase II, which is probably of lysosomal origin, is 90% inhibited by 20 mM L(+) tartrate. Heinrickson [14] separated two or three different species of acid phosphatase from bovine kidney, liver, and pancreas, as well as rat kidney, liver, and spleen, using Sephadex G-75 chromatography. Neil and Horner [15] found that soluble acid phosphatase of guinea-pig liver was inhibited considerably by formaldehyde but only slightly by fluoride and tartrate. On the other hand, lysosomal acid phosphatase is markedly inhibited by fluoride and tartrate but slightly inhibited by formaldehyde. Shibko and Tappel [16] fractionated rat liver cells and reported the acid phosphatase activity of the soluble fraction to be uninhibited by either fluoride or tartrate at the concentrations causing complete inhibition of the lysosomal acid phosphatase. Vanha-Perttula [17] separated rat testicular acid phosphatases (DEAE-cellulose) and studied four distinct forms. The testicular acid phosphatase II seems to be localized in lysosomes and is inhibited by fluoride and tartrate but not by formaldehyde. On the other hand, the enzyme which seems to be of soluble origin, is sensitive to formaldehyde but insensitive to fluoride and tartrate. Miyagawa (unpublished data) has separated two acid phosphatases from extracts of psoriatic scales and rat epidermis, the properties of which were almost the same as for $APase_1$ and $APase_2$ of guinea-pig epidermis.

As mentioned above, lysosomal acid phosphatase from several mammalian organs is markedly inhibited by fluoride and L(+) tartrate but slightly inhibited by formaldehyde. By contrast, soluble acid phosphatase is inhibited by formaldehyde but not sensitive to fluoride and tartrate. The relationship between the data of the present study and those pertaining to other acid phosphatases obtained from several tissues suggests that $APase_1$ is localized in so-called lysosomal particles and $APase_2$ is localized in soluble fractions of guinea-pig epidermis. However, final conclusions regarding the subcellular localization of these enzymes remains open to question until fractionation of the epidermis is established.

Histochemical studies of these enzymes must be done with care since lead nitrate, an inhibitior of $APase_1$, is utilized in the incubation medium of the Gomori technique [18]. Although $APase_2$ is not sensitive to lead nitrate, this form is highly sensitive to the conventional fixatives, formaldehyde and glutaraldehyde.

The authors are greatly indebted to Dr. M. Anai for his review of the manuscript.

REFERENCES

1. Dicken CH, Decker RH: Biochemical evidence for the presence of lysosomes in the epidermis. J Invest Dermatol 47:426–431, 1966
2. Tabachnick J, Perlish JS: Studies on the biochemistry of epidermis. III. Content and some characteristics of 5-nucleotidases, pyrophosphatases and phosphomonoesterases of albino guinea pig and rat epidermis. J Invest Dermatol 48:587–594, 1967
3. Im MJC, Hoppes JE: Alpha-naphthyl acid phosphatase activity in normal human skin and keloids. J Invest Dermatol 57:184–189, 1971
4. Moretti G, Mescon H: Histochemical distribution of acid phosphatases in normal skin. J Invest Dermatol 26:347–360, 1956
5. Hashimoto K, Ogawa K: Histochemical studies on the skin 1. The activity of phosphatase during the histogenesis of the skin in the rat. Am J Anat 113:35–50, 1963
6. Johnson BE, Daniels F: Lysosome and the reactions of the skin to ultraviolet radiation, J Invest Dermatol 53:85–94, 1969
7. Eisen AZ, Arndt KA, Clark WH: The ultrastructural localization of the acid phosphatase in human epidermis. J Invest Dermatol 43:319–326, 1964
8. Prose PH, Sedlis E, Bigelow M: The demonstration of lysosomes in the diseased skin of infants with infantile eczema. J Invest Dermatol 45:448–457, 1965
9. Olson RL, Nordquist RE: Ultramicroscopic localization of acid phosphatase in human epidermis. J Invest Dermatol 46:431–435, 1966
10. Rowden G: Ultrastructural studies of keratinized epithelia of the mouse. I. Combined electron microscope and cytochemical study of lysosomes in mouse epidermis and esophageal epithelium. J Invest Dermatol 49:181–197, 1967
11. Braun-Falco O, Rupec M: Die verteilung der sauren phosphatase bei normaler und psoriatischer verhorung. Dermatologica 134:225–242, 1967
12. Prutkin L, Bogart B: An ultrastructural study of the localization of acid phosphatase activity in the untreated and vitamin A acid treated keratoacanthoma. J Invest Dermatol 54:126–131, 1970
13. DiPietro DL, Zengerle FS: Separation and properties of three acid phosphatases from human placenta. J Biol Chem 242:3391–3396, 1967
14. Heinrikson RL: Purification and characterization of low molecular weight acid phosphatase from bovine liver. J Biol Chem 244:299–307, 1969
15. Neil MW, Horner MW: Studies on acid hydrolases in adult and foetal tissues. Biochem J 92:217–224, 1964
16. Shibko S, Tappel AL: Acid phosphatase of the lysosomal and soluble fraction of rat liver. Biochim Biophys Acta 73:76–86, 1963
17. Vanha-Perttula T: Chromatographic fractionation and characterization of rat testicular acid phosphatase. Biochim Biophys Acta 227:390–401, 1971
18. Gomori G: An improved histochemical technique for acid phosphatase. Stain Technol 25:81–85, 1950